C. R., Ramos, R. J., Burns, D. K., Rumberger, J. M., Rao, B. N. N., Foxall, C., Brandley, B. K. and Lasky, L. A. (1992) J. Cell Biol. **119**, 215–227
19 Hollenbaugh, D., Bajorath, J., Stenkamp, R. and Aruffo, A. (1993) Biochemistry **32**, 2960–2966
20 Rice, K. G., Weisz, O. A., Barthel, T., Lee, R. T. and Lee, Y. C. (1990) J. Biol. Chem. **265**, 18429–18434

Received 16 July 1995

Sialoadhesin and related cellular recognition molecules of the immunoglobulin superfamily

P. R. Crocker†, S. Kelm*, A. Hartnell, S. Freeman, D. Nath, M. Vinson and S. Mucklow
*Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K. and *Biochemisches Institut, University of Kiel, Olshausenstrasse 40, Kiel, Germany*

Introduction

Cell–cell interactions play a crucial role in a broad range of biological and pathological processes encompassing cell migration and differentiation, inflammation, immune function, development and embryogenesis. The majority of cell interaction molecules fall into discrete families based on their primary structure and domain organization. These include the immunoglobulin superfamily, integrins, cadherins and selectins. The largest of these families is the immunoglobulin superfamily (IgSF) which contains over 100 members. These proteins are generally considered to mediate intercellular communication through specific protein–protein interactions. However, the recent characterization of the sialoadhesin family of lectin-like cell adhesion molecules of the IgSF [1,2] has shown that protein–carbohydrate interactions are also important. All members of this family can mediate sialic acid (sia)-dependent adhesion. To date, it includes the eponymous member, sialoadhesin (Sn), expressed uniquely by subpopulations of macrophages [3], CD33 expressed by cells of the myelomonocytic lineage [2,4], CD22 expressed by B-lymphocytes [5,6], the myelin associated glycoprotein (MAG) expressed by myelinating glial cells [1,7] and the Schwann cell myelin protein (SMP) expressed by glial cells of the chick and quail [8,9]. SMP will not be considered further in this article because it is the least characterized of the family and as yet no mammalian homologue has been identified. All members of the sialoadhesin family have a similar Ig domain organization in the extracellular regions, being composed of an N-terminal V-set domain followed by variable numbers of C2-set Ig domains (Table 1).

Recently, Powell and Varki [9] have proposed that all lectins belonging to the IgSF should be called I-type lectins. While this is a useful classification, the similarity in domain organization and binding properties clearly sets the sialoadhesin family apart from other potential IgSF lectins such as N-CAM [10]. We therefore feel that the term 'sialoadhesins' should be used to specifically encompass the molecules Sn, CD22, MAG and CD33.

Biological roles of sialoadhesins

Concerning the functions of sialoadhesins, most information is currently available for CD22 and MAG. CD22 is associated with the B-cell receptor complex and becomes phosphorylated on B-cell activation [11,12]. A recent study [13] has shown that phosphorylated CD22 binds and activates SHP, a protein tyrosine phosphatase that negatively regulates signalling through the B-cell receptor complex. It was suggested that, in lymphoid tissues, ligation of CD22 by high-affinity sialylated ligands on neighbouring lymphocytes could sequester CD22 and its associated SHP tyrosine phosphatase from the B-cell receptor complex, thus lowering the activation threshold to antigen.

For MAG, attention has focused on its potential role in the initiation of myelination since it is phosphorylated on tyrosine residues and can associate with and activate the fyn tyrosine kinase [14–16]. However, in transgenic mice lacking MAG, myelination is essentially normal [17,18] though in adult mice there are profound morphological abnormalities, suggesting an important function for MAG in the maintenance

Abbreviations used: IgSF, immunoglobulin superfamily; sia, sialic acid; Sn, sialoadhesin; MAG, myelin associated glycoprotein; SMP, Schwann cell myelin protein; RBCs, erythrocytes.

†To whom correspondence should be addressed.

of myelin [19]. Studies *in vitro* have also demonstrated that MAG can function in the regulation of axonal growth [20–22]. This can occur both positively and negatively depending on the type and age of the neuronal cell [21,22]. These observations have important implications for the potential role of MAG in neuronal regeneration.

For Sn, its expression pattern and selective interactions with cells of the myelomonocytic lineage suggest a role in regulation of myeloid cell development, either trophic or phagocytic [23,24]. Likewise, the expression pattern and binding properties of CD33 are consistent with a role in haemopoietic regulation [2].

Characterization of the sialoadhesin family

The characterization of the sialoadhesin family came about through independent studies on CD22 and Sn. In the case of CD22, a 'reverse genetics' approach led to its characterization as a sia-dependent lectin. CD22α, a truncated form of CD22 lacking domains 3 and 4, was cloned in 1990 by Stamenkovic and Seed and shown to mediate binding to monocytes and erythrocytes (RBCs) when expressed in COS cells [25]. Wilson et al. [5] cloned the full-length form of CD22 (CD22β) and subsequent studies with this isoform established that it mediated cell adhesion in a sia-dependent manner. A series of elegant studies showed that the minimal structure recognized by CD22β was Siaα2,6Hex(NAc) where Hex(NAc) can be Gal, GalNAc or GlcNAc [6,26,27]. COS cells transfected with CD22β cDNA could bind in a sia-dependent manner to a range of haemopoietic cell types, notably lymphocytes, monocytes, neutrophils and RBCs [28], but with a striking preference for B- and T-lymphocytes [1,24].

In contrast, Sn was originally discovered as a sia-dependent sheep RBC receptor expressed by stromal macrophage populations in the mouse [29]. The subsequent isolation of an inhibitory monoclonal antibody led to its purification and the direct demonstration that Sn is a sia-dependent lectin, with a minimal structural binding requirement of Siaα2,3Gal in either O-glycans, N-glycans or glycolipids [30]. It was subsequently cloned last year and shown to be a new member of the IgSF with 17 extracellular Ig domains which shared sequence similarity with CD22, CD33, MAG and SMP [3].

Neither CD33 [4] nor SMP [8] had previously been shown to function as cell adhesion molecules. However, both purified native MAG and recombinant MAG expressed in L-cells had been shown to interact with heterologous ligands on a range of cell types including neuronal cells, oligodendrocytes and fibroblasts, but the nature of these ligands was not known [31,32]. An obvious possibility, based on the homology with CD22 and Sn, was that MAG was also a sia-dependent lectin and that this could account for the wide range of cell types with which it was known to interact. Human RBCs provide a useful model system to study the specificity of sia-binding proteins because they can be derivatized to carry sialic acids in unique linkages [33]. A series of binding assays was performed using MAG expressed on the surface of COS cells or as a soluble recombinant protein immobilized to polystyrene or aggregated in solution [1]. These assays established that MAG is indeed a sia-dependent lectin with a specificity distinct from that of Sn, preferring the oligosaccharide structure Siaα2,3Galβ1,3GalNAc in O-glycans over Siaα2,3Galβ1,4GlcNAc in N-glycans (Figure 1 and Table 1).

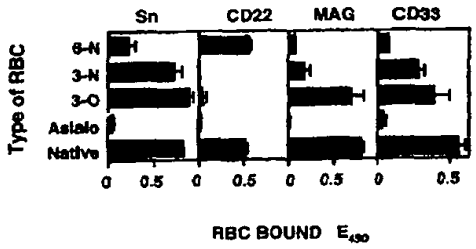
A similar approach was used with CD33 which was also shown to function as a sia-dependent adhesin which immobilized to polystyrene [2]. Its specificity was found to be remarkably similar to that of Sn, preferring Siaα2,3Gal in N- and O-glycans (Figure 1 and Table 1). However, when expressed on the surface of COS cells, CD33 was unable to mediate adhesion unless the transfected COS cells were pretreated with sialidase to remove endogenous ligands [2]. The significance of inhibitory '*cis*-interactions' with carbohydrate ligands is discussed further below.

Structural features and chromosomal localization of sialoadhesins

Homology between sialoadhesins is particularly apparent over the first two to four Ig-like domains where they share about 35% amino acid identity or 50% similarity if conservative substitutions are taken into account [3]. The N-terminal domain of all proteins has the characteristics of a classical V-set Ig domain, with nine predicted β-strands organized into the ABED and GFCC'C" faces (Figure 2). Ig domains usually contain two conserved cysteines in strands B and F which help stabilize the two β-sheets by forming an intersheet disulphide bond [34]. Williams et al. [35] first pointed out that the V-set domain found in sialoadhesins contains an unusual

Immobilized Fc-adhesin constructs of Sn, CD22, MAG and CD33 bind to specific sia-containing glycans

Fc adhesins containing the entire extracellular region (CD33) or the N-terminal 3 (MAG, CD22) or 4 (Sn) Ig domains fused to the Fc region of human IgG1 were immobilized to plastic as described [1]. Human RBCs (native) were treated with *Vibrio cholerae* sialidase (asialo) and reconstituted to give RBCs containing exclusively 3-*O* (Siaα2,3Galβ1,3GalNAc), 3-*N* (Siaα2,3Galβ1,3(4)GlcNAc) or 6-*N* (Siaα2,6Galβ1,4GlcNAc) structures. Binding of RBC populations (E_{450}) was assessed by measuring the pseudoperoxidase activity in the bound RBCs. For further details see [1,2].



arrangement of conserved cysteines. The cysteine normally present on strand F has been substituted with an alternative hydrophobic residue and a conserved cysteine is present on strand E (Figure 2). This would be expected to lie next to the conventional cysteine near the top of strand B of the ABED face and form an intra-sheet disulphide bond. An additional important feature of sialoadhesins is that the first and second domains contain an extra unpaired cysteine. In domain 1 this lies at the bottom of strand B and in domain 2 it is predicted to be within the BC loop at the top of the domain (Figure 2). Previous observations with MAG [36], and more recently with Sn (A. May and P. R. Crocker, unpublished work), provide direct evidence that these cysteines form an interdomain disulphide. To date, this interdomain disulphide is a unique structural feature of the sialoadhesins and could play a role in sia binding, particularly for CD22, MAG and CD33 (see below).

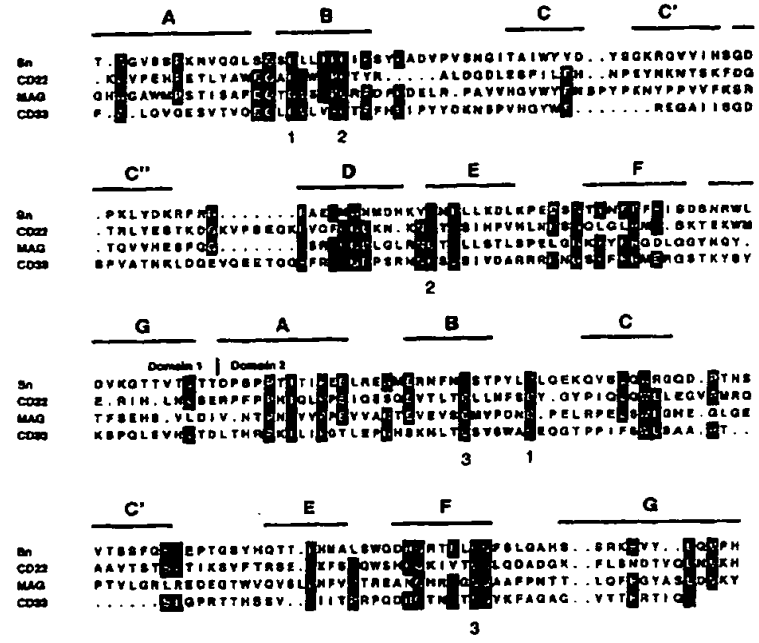
The similarities in sequence and domain organization between Sn, CD22, MAG and CD33 suggest that they evolved from a common ancestral gene, perhaps encoding the first and second Ig-like domains as a minimal sia binding unit. The varying number of C2-set domains could have arisen through gene duplication. Consistent with this idea, it has been found that the *CD22*, *MAG* and *CD33* genes map very closely on

Properties of the Sialoadhesin family

Protein	Domain organization	Chromosomal localization	Expressed by	Minimal oligosaccharide recognized	Sialic acid preference	Cellular preference	Key references
Sn	V $(C2)_{16}$	Human chromosome 20p13 Mouse chromosome 2	Macrophages	Siaα2,3Galβ1,3GalNAc Siaα2,3Galβ1,3(4)GlcNAc	Neu5Ac ≫ Neu5Gc, $Neu5,9(Ac)_2$	Myeloid	[3,24,30,49]
CD22	V $(C2)_6$	Human chromosome 19q13.1–3 Mouse chromosome 7	B-lymphocytes	Siaα2,6Galβ1,4GlcNAc	Murine: Neu5Gc > Neu5Ac	Lymphoid	[1,5,6,28,40,48,49]
MAG	V $(C2)_4$	Human chromosome 19q13.1–3 Mouse chromosome 7	Oligodendrocytes Schwann cells	Siaα2,3Galβ1,3GalNAc	Neu5Ac ≫ Neu5Gc, $Neu5,9(Ac)_2$	Neuronal?	[1,7,37]
CD33	V C2	Human chromosome 19q13.1–3 Mouse chromosome 7	Myeloid cells	Siaα2,3Galβ1,3GalNAc Siaα2,3Galβ1,3(4)GlcNAc	Not determined	Myeloid	[2,4]

Alignment of the N-terminal two Ig domains of Sn, CD22, CD33 and MAG

Sequences are murine Sn, human CD22, murine MAG and human CD33. Alignments were generated using the GCG BESTFIT program and optimized by eye. The predicted β-strand assignments are indicated by bars. Three or more residues that are identical are boxed in black. Conserved cysteines likely to form disulphide bonds are numbered. Disulphide bonds are predicted between domains 1 and 2 (cysteines marked 1), within the ABED β-sheets of the C2-set domain 2 (cysteines marked 3).



human chromosome 19q13.1 and on a syntenic region of mouse chromosome 7, linked to the structurally related genes of the carcinoembryonic and pregnancy-specific glycoprotein families [37–40]. Surprisingly, the *Sn* gene has been mapped recently to a distinct locus, on chromosome 20p in the human and a syntenic region on chromosome 2 in the mouse [41]. This indicates that the *Sn* gene became separated from the related genes, *CD22*, *CD33* and *MAG*, before mammalian speciation. Where studied, the structures of the genes encoding the sialoadhesins are typical of non-neuronal members of the IgSF each Ig domain being encoded by a separate exon [40] (S. Mucklow and P. R. Crocker, unpublished work).

Role of *cis*-interactions in modulation of sia-dependent adhesion

An important feature that is beginning to emerge for members of the sialoadhesin family is that their binding activity, when expressed in the plasma membrane, can be modulated via *cis*-interactions with glycoconjugates in the glycocalyx or expressed on the molecule itself. This has been demonstrated in two separate studies with CD22 [42,43] and one with CD33 [2]. In the case of CD33, we found that when the molecule was expressed in the plasma membrane of COS cells it was unable to bind to cellular ligands, such as RBCs or HL-60 cells, unless the transfected cells were first pretreated with sialidase to destroy endogenous ligands [2]. This cryptic binding of CD33 was specific because it could be completely blocked by Fab fragments from an antiserum raised to CD33.

Although the biological significance of *cis*-interactions with ligands is unclear, it could be an important way of regulating cell–cell interactions. One possibility is that it would only allow interactions with cells bearing high-affinity ligands which could displace endogenous low-

affinity ligands from the receptor. Ultimately these interactions would be regulated through expression of the appropriate glycosyltransferases both in receptor-bearing cells and ligand-bearing cells. In the case of B-cells and CD22, for example, the expression of the α2,6 sialyltransferase is upregulated on activation [44] and would result in expression of ligands on B-cells that could interact with CD22 in *cis* [42,43]. This could lead to decreased interaction with ligands on neighbouring cells and could be important in regulating B-cell responses to antigen [13]. For CD33, expression of glycosyltransferases at specific steps in the differentiation pathway of myeloid cells could be important in allowing cell–cell interactions within the haemopoietic microenvironment.

The phenomenon of inhibitory *cis*-interactions for CD22 and CD33 immediately offers an interesting explanation for why sialoadhesin has evolved such a large number of extracellular domains [3]. In the so-called 'rainforest model', we propose that by extending the binding region of the molecule outside of the glycocalyx, this would help avoid the potentially inhibitory effect of *cis*-interactions with sialylated ligands and allow sialoadhesin to remain in a constitutively active state. Rotary shadowing experiments have shown that sialoadhesin is an extended structure of about 50 nm [30], a length which would be required to extend out of the glycocalyx and function in cellular interactions when present in its natural environment.

In addition to the potentially inhibitory effect of the glycocalyx, the presence of inhibitors in extracellular fluids should also be considered. For CD22, the IC_{50} for 2,6-sialyllactose is around 50 μm [27], whereas the concentration of α2,6-linked sia in human plasma is around 1 mM [45], thereby making it very unlikely that CD22 can function in intercellular interactions in the vascular compartments [9]. In contrast, the affinity of sialoadhesin for sia appears to be considerably lower, since 2,3-sialyllactose has an IC_{50} of around 2 mM [24,29] and the concentration of α2,3-linked sia in plasma is reported to be around 0.5 mM [45]. This indicates that sialoadhesin is likely to be able to function in plasma-rich environments such as the bone marrow, liver and spleen.

One of the challenges for the future is to identify the high-affinity ligands for members of the sialoadhesin family. Considerable progress has already been made for CD22 which has been shown to recognize several glycoproteins expressed on the surface of lymphocytes, the most notable of which is the CD45 antigen [46,46a].

Localization of the binding site in sialoadhesin and CD22

A major aim is to understand the molecular basis of sia recognition by sialoadhesins. This is likely to be distinct from the nature of sia recognition by selectins, the other major class of mammalian sia binding proteins, because modifications of sia, such as mild periodate oxidation, have no effect on selectin binding but drastically impair recognition by sialoadhesins [6,29,46]. For selectins, sia can be replaced by sulphate, showing that a negative charge in the correct position is sufficient for recognition [47]. In contrast, sialoadhesins require specific structural components of sia, as shown by the fact that naturally occurring variants of sia at the 5- and 9-carbon positions are differentially recognized [48,49] (Table 1).

In order to identify the domain(s) of Sn and CD22 involved in sia-dependent adhesion, we generated truncated forms of the molecules fused to the Fc portion of human IgG1. These contained either the entire extracellular region or varying numbers of N-terminal domains. In a range of adhesion assays, it was clear that the first N-terminal domain of Sn was both necessary and sufficient to mediate sia-dependent binding of the correct specificity [46a]. In contrast, CD22 required the presence of domains 1 and 2 to mediate binding. Similar observations have been made with CD33 (S. D. Freeman, unpublished work). Taken together, these results suggest that the binding site of sialoadhesins is in domain 1, but for CD22, CD33 and possibly MAG, the second domain is required for correct folding. This is analogous to findings made with intercellular adhesion molecules and CD4, for which the binding sites are located in the first domain but the second domain is needed for correct folding [50].

Future perspectives

Rapid progress has been made over the last 2 years in characterizing the sialoadhesin family as a distinct subgroup of the IgSF that uses sialylated glycoconjugates as specific recognition determinants in a wide range of biological processes. Major questions that lie ahead are to understand the precise functions of these mole-

cules, their roles in cell–cell interactions and signalling and the significance of *cis*-interactions with carbohydrate ligands. It is anticipated that the creation of transgenic mice lacking sialoadhesins will help in these respects, as already demonstrated with MAG. Ectopic expression of sialoadhesins in transgenic animals may also give valuable insights.

The minimal oligosaccharide structures recognized by sialoadhesins are commonly found on cell surfaces and in extracellular fluids. Therefore, it will be important to understand how sialoadhesins can discriminate high-affinity ligands from the excess of low-affinity ligands in their microenvironments. Clearly, the structural elucidation of the sia binding site in sialoadhesins is essential for this and will be a major future goal.

1 Kelm, S., Pelz, A., Schauer, R., Filbin, M. T., Tang, S., Bellard, M.-E. D., Schnaar, R. I., Mahoney, J. A., Hartnell, A., Bradfield, P. and Crocker, P. R. (1994) Curr. Biol. **4**, 965–972

2 Freeman, S. D., Kelm, S., Barber, E. K. and Crocker, P. R. (1995) Blood **85**, 2005–2012

3 Crocker, P. R., Mucklow, S., Bouckson, V., McWilliam, A., Willis, A. C., Gordon, S., Milon, G., Kelm, S. and Bradfield, P. (1994) EMBO J. **13**, 4490–4503

4 Simmons, D. and Seed, B. (1988) J. Immunol. **141**, 2797–2800

5 Wilson, G. L., Fox, C. H., Fauci, A. S. and Kehrl, J. H. (1991) J. Exp. Med. **173**, 137–146

6 Powell, L. D., Sgroi, D., Sjoberg, E. R., Stamenkovic, I. and Varki, A. (1993) J. Biol. Chem. **268**, 7019–7027

7 Salzer, J. L., Holmes, W. P. and Colman, D. R. (1987) J. Cell. Biol. **104**, 957–965

8 Dulac, C., Tropak, M. B., Cameron-Curry, P., Rossier, J., Marshak, D. R., Roder, J. and Dourin, N. M. L. (1992) Neuron **8**, 323–334

9 Powell, L. D. and Varki, A. (1995) J. Biol. Chem. **270**, 14243–14246

10 Horstkorte, R., Schachner, M., Magyar, J. P., Vorherr, T. and Schmitz, B. (1993) J. Cell Biol. **121**, 1409–1421

11 Schulte, R. J., Campbell, M. A., Fischer, W. H. and Sefton, B. M. (1992) Science **258**, 1001–1004

12 Leprince, C., Draves, K. E., Geahlen, R. L., Ledbetter, J. A. and Clark, E. A. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 3236–3240

13 Doody, G. M., Justement, L. B., Delibrias, C. C., Mathews, R. J., Lin, J. J., Thomas, M. L. and Fearon, D. T. (1995) Science **269**, 242–244

14 Bambrick, L. L. and Braun, P. E. (1991) Dev. Neurosci. **13**, 412–416

15 Jaramillo, M. L., Afar, D. E., Almazan, G. and Bell, J. C. (1994) J. Biol. Chem. **269**, 27240–27245

16 Umemori, H., Sato, S., Yagi, T., Aizawa, S. and Yamamoto, T. (1994) Nature (London) **367**, 572–576

17 Li, C., Tropak, M. B., Gerlai, R., Clapoff, S., Abramow Newerly, W., Trapp, B., Peterson, A and Roder, J. (1994) Nature (London) **369**, 747–750

18 Montag, D., Giese, K. P., Bartsch, U., Martini, R., Lang, Y., Bluthmann, H., Karthigasan, J., Kirschner, D. A., Wintergerst, E. S., Nave, K. A., Zielasek, J., Tokya, K. V., Lipp, H. P. and Schachner, M. (1994) Neuron **13**, 229–246

19 Fruttiger, M., Montag, D., Schachner, M. and Martinin, R. (1995) Eur. J. Neurosci. **7**, 511–515

20 Johnson, P. W., Abramow Newerly, W., Seilheimer, B., Sadoul, R., Tropak, M. B., Arquint, M., Dunn, R. J., Schachner, M. and Roder, J. C. (1989) Neuron **3**, 377–385

21 McKerracher, L., David, S., Jackson, D. L., Kottis, V., Dunn, R. J. and Braun, P. E. (1994) Neuron **13**, 805–811

22 Mukhopadhyay, G., Shen, Y.-J., Doherty, P., Walsh, F., Crocker, P. and Filbin, M. (1994) Neuron **13**, 757–767

23 Crocker, P. R., Werb, Z., Gordon, S. and Bainton, D. F. (1990) Blood **76**, 1131–1138

24 Crocker, P., Freeman, S., Gordon, S. and Kelm, S. (1995) J. Clin. Invest. **95**, 635–643

25 Stamenkovic, I. and Seed, B. (1990) Nature (London) **345**, 74–77

26 Powell, L. D. and Varki, A. (1994) J. Biol. Chem. **269**, 10628–10636

27 Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S. and Varki, A. (1995) J. Biol. Chem. **270**, 7523–7532

28 Engel, P., Nojima, Y., Rothstein, D., Zhou, L.J., Wilson, G. L., Kehrl, J. H. and Tedder, T. F. (1993) J. Immunol. **150**, 4719–4732

29 Crocker, P. R. and Gordon, S. (1986) J. Exp. Med. **164**, 1862–1875

30 Crocker, P. R., Kelm, S., Dubois, C., Martin, B., McWilliam, A. S., Shotton, D. M., Paulson, J. C. and Gordon, S. (1991) EMBO J. **10**, 1661–1669

31 Afar, D. E., Marius, R. M., Salzer, J. L., Stanners, C. P., Braun, P. E. and Bell, J. C. (1991) J. Neurosci. Res. **29**, 429–436

32 Sadoul, R., Fahrig, T., Bartsch, U. and Schachner, M. (1990) J. Neurosci. Res. **25**, 1–13

33 Paulson, J. C. and Rogers, G. N. (1987) Methods Enzymol. **138**, 162–168

34 Williams, A. F. and Barclay, A. N. (1988) Annu. Rev. Immunol. **6**, 381–405

35 Williams, A. F., Davis, S. J., He, Q. and Barclay, A. N. (1989) Cold Spring Harbor Symp. Quant. Biol. **54**, 637–647

36 Pedraza, L., Owens, G. C., Green, L. A. D. and Salzer, J. L. (1990) J. Cell Biol. **111**, 2651–2661

37 Barton, D. E., Arquint, M., Roder, J., Dunn, R. and

Francke, U. (1987) Genomics **1**, 107–112
38 Law, C. L., Torres, R. M., Sundberg, H. A., Parkhouse, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A. and Clark, E. A. (1993) J. Immunol. **151**, 175–187
39 Peiper, S., Ashmun, R. and Look, A. (1988) Blood **72**, 314–321
40 Wilson, G. L., Najfeld, V., Kozlow, E., Menniger, J., Ward, D. and Kehrl, J. H. (1993) J. Immunol. **150**, 5013–5024
41 Mucklow, S., Hartnell, A., Mattei, M. G., Gordon, S. and Crocker, P. R. (1995) Genomics **28**, 344–346
42 Braesch-Andersen, S. and Stamenkovic, I. (1994) J. Biol. Chem. **269**, 11783–11786
43 Hanasaki, K., Varki, A. and Powell, L. D. (1995) J. Biol. Chem. **270**, 7533–7542
44 Erikstein, B. K., Funderud, S., Beiske, K., Aaseng, A., Davies, C. D., Blomhoff, H. K. and Smeland, E. B. (1992) Eur. J. Immunol. **22**, 1149–1155
45 Hanasaki, K., Powell, L. D. and Varki, A. (1995) J. Biol. Chem. **270**, 7543–7550
46 Sgroi, D., Varki, A., Braesch-Anderson, S. and Stamenkovic, I. (1993) J. Biol. Chem. **268**, 7011–7018
46a Nath, D., van der Merwe, P. A., Kelm, S. and Crocker, P. R. (1995) J. Biol. Chem., in the press
47 Green, P. J., Tamatani, T., Watanabe, T., Miyasaka, M., Hasegawa, A., Kiso, M., Yuen, C. T., Stoll, M. S. and Feizi, T. (1992) Biochem. Biophys. Res. Commun. **188**, 244–251
48 Sjoberg, E., Powell, L., Klein, A. and Varki, A. (1994) J. Cell Biol. **126**, 549–562
49 Kelm, S., Schauer, R., Manuguerra, J.-C., Gross, H.-J. and Crocker, P. R. (1994) Glycoconjugate J. **11**, 576–585
50 Berendt, A. R., McDowall, A., Craig, A. G., Bates, P. A., Sternberg, M. J., Marsh, K., Newbold, C. I. and Hogg, N. (1992) Cell **68**, 71–81

Received 18 August 1995

C-type lectins of natural killer cells: carbohydrate ligands and role in tumour cell lysis

K. Bezouška

Department of Biochemistry, Faculty of Science, Charles University Prague, Hlavova 8, CZ-12840 Praha 2, Czech Republic

Introduction

Natural killer (NK) cells constitute a distinct lymphocyte subset defined by the absence of B- or T-cell surface receptors, and characterized by their natural cytotoxicity against certain tumour or virally infected cells [1]. As physical contact is required for this spontaneous cytotoxicity, the molecular basis for NK cell reactivity should involve effector (NK) cell membrane receptors engaging their specific ligands at the target (tumour) cell surface. Type II integral membrane proteins that contain extracellular domains related to the carbohydrate-recognition domain (CRD) of C-type animal lectins have recently been identified among candidates for NK cell receptor functions [2,3]. Here I summarize our recent investigations aimed at identifying the ligands for these receptors. These include high-affinity oligosaccharide ligands of the type represented on the tumour cell surface that are recognized by rat NKR-P1 or similar receptors at the NK cell surface and are intimately linked to natural killing. Unpublished work is also presented, pointing to the possible involvement of one of these molecules, rat NKR-P1, in adhesive reactions of NK cells.

Abbreviations used: NK, natural killer; CRD, carbohydrate-recognition domain; IL-2, interleukin-2.

NK cell receptors related to Ca^{2+}-dependent animal lectins

In recent years, extensive experimental efforts of immunologists have been concentrated on the identification of NK cell receptors, employing both hybridoma technology (production of monoclonal antibodies against purified NK cell populations) and molecular genetic approaches (such as subtractive hybridization) [3–6]. Both strategies have ultimately converged in the identification of a group of type II disulphide-linked dimeric transmembrane proteins related to Ca^{2+}-dependent animal lectins [3]. These molecules, the protein products of polymorphic gene families residing in the natural killer gene complex in the distal region of mouse chromosome 6 or the syntenic human chromosome 12p13.2 [2,3], have been assigned to a separate group (group V, Figure 1*a*) in the evolutionary tree of this lectin superfamily [7]. They are notable among C-type lectins by possessing, within their intracellular tails, peptide sequences that seem to enable them to transduce signals to the NK cell upon cross-linking with antibodies or engage-